



开启生命程序: 合子基因组如何启动?

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摘要 合子基因组激活(zygotic genome activation, ZGA)是卵子受精后的第一个转录事件, 标志着胚胎基因表达程序的启动. ZGA缺陷严重影响胚胎发育, 甚至导致胚胎死亡. 随着微量细胞测序技术和单细胞分析技术的快速发展, 人们对ZGA的理解在近年来取得了长足进步. 相关研究揭示了ZGA期间表观基因组、转录组、蛋白质组和代谢组的景观、重编程过程和相关机制. 此外, ZGA的正常启动依赖多方面因素的协同调控. 表观遗传重编程为ZGA及后续转录过程建立适宜的染色质环境, ZGA也影响表观遗传的重编程. ZGA也广泛受到RNA水平的调控, 关键转录因子通过选择性激活基因表达驱动ZGA发生. 本综述阐述了不同物种ZGA相关表观遗传重编程事件, ZGA相关RNA调控因子、核心ZGA转录因子的作用机制, 以及ZGA期间转录组、蛋白质组、代谢组等多维度重编程景观. 最后, 我们针对ZGA过程中尚未解决的科学问题进行了深入探讨.

关键词 合子基因组激活(ZGA), 表观遗传, 转录因子, 胚胎发育

在迄今为止已研究的所有物种中, 合子基因组在受精后都存在一段转录沉默时期^[1]. 随后, 随着母源mRNA的清除和合子基因组的激活(zygotic gene activation, ZGA), 胚胎进入全能性状态^[2]. ZGA有两个转录波峰, 第一次波峰规模较小, 被称为次要ZGA(minor ZGA)^[3]; 第二次波峰规模较大, 被称为主要ZGA(major ZGA)^[4]. 通常认为, 小鼠的次要ZGA发生在1细胞晚期到2细胞早期, 主要ZGA发生在2细胞晚期^[5]; 人类大规模ZGA主要发生在4~8细胞期^[6]. 也有研究表明, 人类胚胎中在1细胞期就能被检测到转录^[7]. ZGA期间, 染色质状态会发生大规模重编程, 以适应这种剧烈的转录事件^[8]. 关键的转录因子(transcription factors, TFs)在卵母细胞中以母源mRNA的形式存在, 或在ZGA早期转

录, 通常被视为ZGA发生的驱动力, 以实现选择性的基因激活^[9]. 本文首先阐述ZGA期间的表观遗传重编程和染色质重塑事件, 然后回顾RNA修饰、RNA剪接以及lncRNA对ZGA的影响, 并总结不同物种中关键的ZGA转录因子及其对胚胎发育的功能, 最后我们对翻译组、蛋白组和代谢组在ZGA期间的变化及其在ZGA过程中的作用进行讨论.

1 ZGA期间的表观遗传重编程和染色质重塑

激活性染色质修饰、抑制性染色质修饰、三维基因组结构变化、组蛋白变体和RNA分子在ZGA过程中发生动态变化, 发挥重要的调控功能.

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1.1 激活性染色质修饰

组蛋白乙酰化通常作为活跃启动子和增强子的标志,可以促进染色质开放,增强转录活性^[10].其中,H3K27ac是一个被广泛研究的组蛋白乙酰化修饰.在ZGA发生前的斑马鱼^[11]、果蝇^[12]和小鼠^[13]胚胎中均发现启动子附近存在H3K27ac修饰.在小鼠中,ZGA前后的乙酰化经历了三波主要的全局转变.生发泡期(germinal vesicle, GV)卵母细胞的高乙酰化首先转变为MII卵母细胞的低乙酰化,随后在合子中重新转变为高乙酰化.在GV卵母细胞和合子中,H3K27ac呈现出非经典的分布模式:其分布广泛且呈现为宽峰,和H3K4me3分布重叠,并与染色质可及性正相关.H3K27ac在2细胞期被重编程为经典的分布模式^[14](图1).在人类中,H3K27ac在2细胞和4细胞中呈现出类似的非经典分布模式,并在8细胞期重编程为经典的分布模式^[15].在成熟精子中,尽管大多数组蛋白被鱼精蛋白取代,但剩余的组蛋白仍被广泛修饰^[16].过去认为小鼠成熟精子中组蛋白修饰模式和体细胞高度相似.最近的研究指出,先前的结果可能受到了体细胞来源游离染色质的污染,精子中组蛋白修饰情况有待进一步研究^[17].受精后,父源基因组的组蛋白修饰迅速减少,这可能与鱼精蛋白-组蛋白交换有关^[18].

斑马鱼中,对组蛋白乙酰转移酶(histone acetyltransferase, HAT) P300或组蛋白乙酰化修饰的读取者(reader) BRD4进行化学抑制,或敲低其他组蛋白乙酰转移酶ep300b、crebbpa和crebbpb均会导致合子转录下调和ZGA缺陷^[11,19].将P300人工招募到ZGA基因的启动子和增强子上,可在无转录因子的情况下直接激活ZGA基因表达^[20].以上研究表明组蛋白乙酰化是ZGA中转录激活的充要条件.类似地,在小鼠中抑制CBP/P300会导致小鼠ZGA缺陷、染色质可及性全局下降和2细胞期发育阻滞^[14].抑制组蛋白去乙酰化酶(histone deacetylase, HDAC)活性也会导致发育停滞和某些发育基因的异常表达^[14].有研究表明,通过烟酰胺腺嘌呤二核苷酸(NAD^+)去除组蛋白乙酰化是小鼠次要ZGA正常退出的关键^[21].综上所述,组蛋白的乙酰化和去乙酰化对ZGA都至关重要.

除乙酰化修饰外,组蛋白赖氨酸残基还能发生其他多种酰化修饰^[22].其中,巴豆酰化修饰通常发生在活跃启动子和增强子上,可以增强转录活性^[23].ZGA期间的巴豆酰化修饰由P300介导,该酶同时具有HAT和巴

豆酰化转移酶(histone crotonyltransferase, HCT)活性.在小鼠中敲低p300导致巴豆酰化水平下降和胚胎8细胞期阻滞.在P300敲除胚胎中回补丧失HAT活性但保留HCT活性的P300突变体可恢复巴豆酰化水平,约50%胚胎能够成功发育到囊胚期^[24].

H3K4me3通常作为活跃启动子的组蛋白修饰标记,可以帮助募集染色质重塑因子和转录机器^[25].果蝇胚胎在ZGA发生之前没有检测到H3K4me3^[26].在斑马鱼^[27]和小鼠^[13]中,H3K4me3在ZGA之前就已经存在.在小鼠、大鼠、牛和猪的卵母细胞中,H3K4me3以非经典的宽峰形式(non-canonical H3K4me3)存在于启动子和许多远端调控区^[13,28].非经典H3K4me3所覆盖的远端区域大部分属于部分甲基化区域(partially methylated domains, PMDs)^[28].通过过表达组蛋白去甲基化酶KDM5B或敲除甲基转移酶KMT2B以擦除H3K4me3会导致原本沉默的成熟卵母细胞重新激活转录,受精后的胚胎发育阻滞在1~4细胞期^[28].这些结果提示H3K4me3可能在小鼠卵母细胞的基因组沉默中发挥非经典功能.H3K4me3在2细胞期逐渐转为经典的分布模式^[13,28],这种转变部分依赖于ZGA,由于参与转变的组蛋白去甲基化酶KDM5A/5B由ZGA基因编码^[13].敲除*Kdm5a/5b*会导致大量ZGA基因表达下调和囊胚期发育阻滞^[13],提示H3K4me3的模式转换可能对小鼠胚胎发育有重要功能.然而,人类卵母细胞和胚胎中并不存在非经典H3K4me3.仅在4细胞期,富含CG的染色质开放区域会短暂出现H3K4me3宽峰^[29].因此,非经典H3K4me3及其模式转变的功能有待进一步研究.

1.2 抑制性染色质修饰

DNA甲基化在转录抑制、基因印记、X染色体失活以及转座元件的抑制等过程中都发挥了重要作用^[30].在小鼠精子中,DNA甲基化水平约为80%,相对均匀地覆盖了大部分基因组.相反,成熟小鼠卵母细胞中呈现基因组高低相间的甲基化模式,且高度甲基化区域集中在转录区域^[31].这种转录依赖性DNA甲基化的一个重要功能是帮助卵母细胞建立基因印迹^[32].在小鼠中,这种甲基化是通过DNA甲基转移酶DNMT3A和DNMT3L介导的.

在小鼠和人类胚胎中,合子受精后即发生大规模的全局DNA去甲基化(图1).这一过程持续到囊胚阶段,在囊胚中仅剩余20%~40%的CpG位点保留配子遗传的甲基化^[33].这些保留高度甲基化的CpG位点包括印记

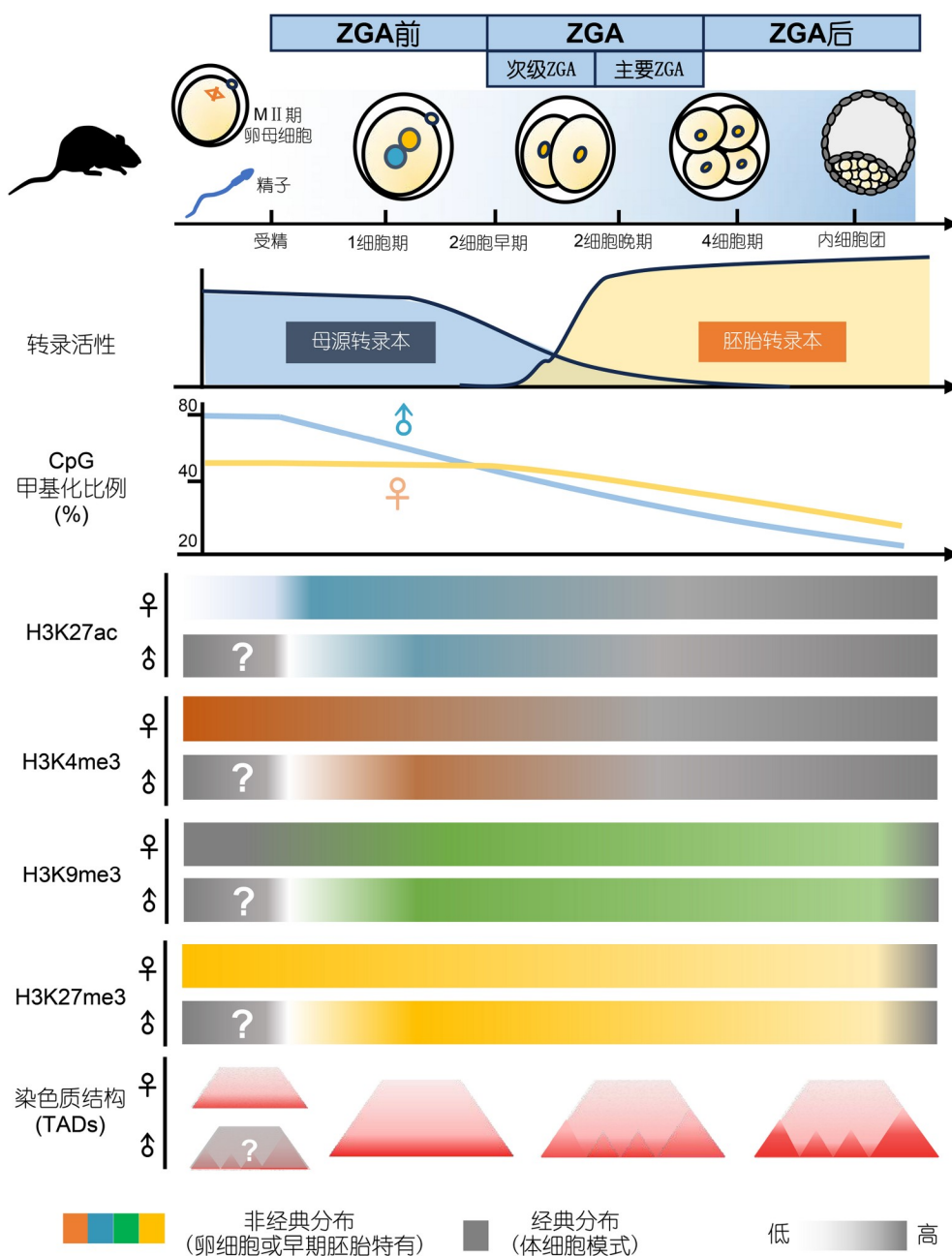


图 1 小鼠ZGA前后的表观基因景观重编程。在ZGA期间，卵细胞基因表达模式转变为胚胎基因表达模式。受精后，母源和父源基因组即发生大规模全局去甲基化，父源基因组甲基化水平下降更剧烈。母源基因组的非经典H3K4me3和H3K27me3(除启动子处的H3K27me3外)是由卵母细胞继承而来。父源基因组的组蛋白修饰在受精后大部分擦除并重新建立。H3K4me3和H3K27ac在2细胞期依赖ZGA重编程为经典模式，非经典的H3K9me3和H3K27me3一直保留到囊胚阶段。在MII卵母细胞中，染色质呈现典型的细胞分裂中期结构，缺乏TAD。在精子中，组蛋白修饰和染色质三维结构的变化情况还存在争议。受精后，染色质高级结构变得高度松弛，并在植入前的发育过程中逐渐重建

Figure 1 Reprogramming of the Epigenetic Landscape Before and After Mouse ZGA. During ZGA, the transcriptome shifts from maternally inherited transcripts to those transcribed from the embryonic genome. After fertilization, large-scale global DNA demethylation occurs on both the maternal and paternal genomes, with a more pronounced decrease on the paternal genome. Non-canonical H3K4me3 and H3K27me3 (excluding H3K27me3 at promoters) on the maternal genome are inherited from oocytes, whereas histone modifications on the paternal genome are often erased and re-established after fertilization. H3K4me3 and H3K27ac are reprogrammed to canonical patterns at the 2-cell stage in a ZGA-dependent manner. Non-canonical H3K9me3 and H3K27me3 persist until the blastocyst stage. In MII oocytes, chromatin exhibits typical metaphase structure and lacks TAD. The states of histone modifications and three-dimensional chromatin structure in sperm remain controversial. Post-fertilization, the chromatin organization becomes highly relaxed and is gradually restored during pre-implantation development

控制区(imprinting control region, ICR)和某些重复序列^[34]。受精后的全局DNA去甲基化是主动和被动机制共同作用的结果。一方面,母源蛋白STELLA和NLRP14将维持性DNA甲基化酶DNMT1及其辅助因子UHRF1锁定在细胞质中,避免其入核,使得基因组甲基化标记随着DNA的复制逐渐稀释^[35]。另一方面,母源蛋白TET3主动擦除基因组的DNA甲基化。在缺乏STELLA的卵母细胞中,DNMT1/UHRF1发生异常核滞留与卵子全基因组水平的过度DNA甲基化,导致受精后合子基因的激活受阻^[36]。在母源NLRP14缺失的受精卵中也出现DNMT1/UHRF1的异常核定位,基因组去甲基化无法正常完成^[35]。母源NLRP14缺失的受精胚胎还表现出紊乱的钙振荡^[37],提示NLRP14具有独立于DNA甲基化的其他功能。

胚胎基因组在受精后至囊胚期发生全局去甲基化的同时,少数区域建立了从头甲基化。小鼠胚胎从2细胞早期开始,少数父本区域发生DNMT3A依赖的从头甲基化。这些从头甲基化的缺失会导致父本基因过早激活^[38]。人类胚胎在雄原核早期到中期及4~8细胞期发生了两波DNMT3A依赖的从头甲基化过程,这些甲基化倾向于建立在转座元件上,可能与抑制其转座活性和维护基因组稳定性有关^[39]。综上所述,精确的DNA甲基化重编程对于ZGA发生及胚胎发育的正常进行至关重要。

H3K27me3和H2AK119ub是分别由多梳复合体2(polycomb repressive complex 2, PRC2)和多梳复合体1(PRC1)催化的抑制性组蛋白修饰,它们在调控基因表达、基因组印记、X染色体失活和染色质结构方面发挥着关键作用^[40]。在小鼠中,受精后*Hox*等发育基因启动子上的H3K27me3被大量擦除^[41](图1)。然而,母源基因组上的远端H3K27me3仍然被保留,能够沉默包括*Xist*在内的少数基因,同时父源等位基因表达,从而实现不依赖于DNA甲基化的非经典基因印记^[41,42]。与之相反,H2AK119ub在整个小鼠发育过程中始终存在于发育基因的启动子上^[37]。胚胎着床后,H3K27me3和H2AK119ub1的分布耦合,主要出现在发育相关基因的启动子区域^[43]。PRC2关键组分EED和EZH2的母源缺失导致小鼠植入前胚胎中H3K27me3印记的缺失和相应印记基因的双等位基因表达。这些基因的异常表达对囊胚形成没有明显影响,但植入后胚胎会出现发育阻滞^[44]。在合子中去除H2AK119ub会导致部分晚期发育基因在ZGA期间表达上调及4细胞期发育阻滞^[37],这

表明在H3K27me3缺失的情况下,H2AK119ub有助于防止基因的提前表达。另一方面,小鼠卵母细胞中H2AK119ub会在生发泡破裂(germinal vesicle breakdown, GVBD)时发生大规模下调,并在1细胞期上调。其中,下调过程主要由去泛素化酶USP16介导。去泛素化酶USP16的母源缺失导致H2AK119ub在卵子中的异常维持,但不影响卵子成熟。受精后,USP16缺失胚胎出现ZGA异常和出生小鼠数目的降低^[45]。以上研究表明,小鼠早期胚胎中H2AK119ub重编程对ZGA的正常进行至关重要。

与小鼠不同,人ZGA期间发生H3K27me3全局性擦除,在8细胞胚胎中几乎检测不到H3K27me3^[29]。与之相对应,人卵母细胞和ZGA前胚胎中不表达PRC2的核心组分EED和SUZ12^[41]。相应地,人类早期胚胎中*XIST*从两个等位基因中都能转录,并且X染色体基因保持双等位基因表达^[46]。H3K27me3介导X染色体印记失活的现象主要在啮齿类中观察到。

H3K9me3是一种抑制性组蛋白修饰,主要出现在组成型异染色质(constitutive heterochromatin)^[47]。在小鼠中,许多ZGA基因(如*Zscan4*)在MⅡ卵母细胞中具有高水平的H3K9me3,但在受精后被迅速擦除,以便于在2细胞期激活表达^[48]。ZGA发生前H3K9me3的积累可能是ZGA基因在已有RNA聚合酶Ⅱ(polⅡ)预配置的情况下仍不会提前转录的重要原因。4细胞期后,大量H3K9me3在富含长末端重复序列(long terminal repeated, LTR)的区域逐渐建立,导致LTR相关基因表达沉默^[48]。植入前胚胎中的H3K9me3处于较低水平,植入后H3K9me3逐渐重建。受精后H3K9me3的擦除失败经常发生在体细胞核移植(somatic cell nuclear transfer, SCNT)胚胎中,导致ZGA期间基因激活受到影响,可以通过表达去甲基化酶*Kdm4b/d/e*或敲除供体细胞中的甲基转移酶*Suz39h1/2*进行挽救^[49,50]。在卵母细胞中去除去甲基化酶KDM4A会导致H3K9me3的异常积累,进而影响ZGA期间基因和转座元件的正常激活,胚胎在植入前致死^[51]。另一方面,*Suz39h1*在小鼠胚胎中的提前表达会过早建立组成型异染色质,导致胚胎在桑椹胚之后死亡^[52]。在果蝇^[53]、斑马鱼^[54]和爪蟾^[55]中,囊胚期前的细胞周期通常很短,使得组蛋白甲基转移酶SETDB1在快速分裂中被稀释,H3K9me3被擦除。在囊胚期,胚胎分裂速度逐渐减慢,SETDB1浓度和H3K9me3水平随之回升^[54]。这种依赖细胞分裂的调节方式使得这些动物能有效地在ZGA发生前下调

H3K9me3水平。H3K9me3在受精后的擦除可能可以实现部分早期胚胎发育基因在大部分发育时期沉默,但在特异发育时期激活。此外,这可能也是亲代表观遗传记忆的擦除的方式之一。

1.3 组蛋白变体

组蛋白变体是同一组蛋白家族中分化出了氨基酸序列、表达模式或染色质定位存在差异的成员。组蛋白变体和组蛋白共同参与表观遗传调控^[56]。在后生动物中,生殖细胞中存在许多特异的组蛋白H1变体,且雌性生殖细胞特异的H1变体通常保留到胚胎早期。在果蝇^[57]、爪蟾^[58]和小鼠^[59]中,H1胚胎变体在主要ZGA期间被其对应的体细胞类型取代。在果蝇中,缺乏胚胎类型的dBigH1变体会导致ZGA提前,胚胎在细胞化时期发育停滞^[57]。在斑马鱼的精子和早期胚胎中,富含组蛋白变体H2A.Z及H3K4me1修饰的“占位”核小体(placeholder nucleosomes)占据了DNA低甲基化区域。“占位”核小体在早期胚胎中能够帮助指导母源基因组甲基化重编程,使得数百个早期发育和种系特征相关的重要位点转变为低DNA甲基化状态,帮助两个亲代基因组的DNA甲基化模式重编程到相同状态^[60]。在合子中去除H2A.Z导致胚胎在4hpf时期致死^[60]。在小鼠中,H2A.Z在配子中积累,而后在MII卵母细胞、合子和早期2细胞期间被逐渐移除,并在2细胞胚胎中开始重新积累^[61]。siRNA敲低H2A.Z可显著减少小鼠囊胚的形成率,但不会影响ZGA^[61]。

在小鼠配子和早期胚胎中,H2A是变体多样性最高的核心组蛋白^[62]。两种变体H2A.X和TH2A大量出现在GV卵母细胞和合子的核小体中^[62]。其中H2A.X参与细胞中的DNA损伤应答(DNA damage response,DDR)^[63]。早期胚胎中大量的H2A.X确保基因组在快速复制时保持稳定^[64]。大量的H2A.X还能通过抑制H1连接组蛋白的结合维持染色质松弛,促进DDR相关分子的结合^[64]。敲除H2A.X显著降低了辐射胁迫下合子发育到囊胚期的速度,并观察到更多的微核数^[64]。

H3.3作为最保守的H3变体之一存在于启动子、基因体和顺式调控元件中,通常在转录激活中发挥作用^[65]。除此之外,H3.3也沉积在端粒、着丝粒异染色质和沉默的逆转录元件上,表明其在转录调控中的作用依赖于染色质环境^[65]。在小鼠成熟卵母细胞和合子中,H3.3是GV卵母细胞和合子中的主要变体,并呈现出非经典的分布模式,即在活性染色质区域外广泛分布^[66]。

一种可能的解释是,H3.3通过非复制依赖(replication-independent)的方式掺入到核小体中,而H3.1/H3.2以复制依赖(replication-dependent)的方式掺入。因此处于减数分裂I期没有DNA复制的卵母细胞以H3.3为主要H3成分^[66]。这种非经典的分布模式在随后的2细胞期迅速转变为经典模式,H3.3重新出现在启动子区或开放染色质中。有趣的是,在果蝇中似乎出现了相反的趋势,即由卵母细胞提供的经典H3构成了早期胚胎中的大部分H3。直到ZGA前后,H3.3才逐渐增加,伴随着经典H3的减少^[67]。研究表明,H3在H3.3缺失时可以部分补偿H3.3的功能,通过DNA复制非依赖方式装载到染色质上^[68]。

1.4 染色质三维结构

受精后普遍松弛的染色质高级结构是早期胚胎染色质的典型标志。这种现象在不同物种中高度保守^[69],包括果蝇^[70]、斑马鱼^[71]、爪蟾^[72]、小鼠^[66,73]和人类^[74]。染色质三维结构可分为拓扑关联域(topologically associating domain, TAD)、染色质环(loop)和A/B区室(A/B compartment)等多个层次。TAD是内部高度互作,但与外部互作很少的一段染色质连续区域,被认为是染色质空间构象的基本单元^[75]。Loop大部分是由黏连蛋白(cohesin)和CTCF锚定形成的环状结构,loop锚定区域通常富含调控元件^[76]。A compartment对应染色质开放区域,与基因富集处、高转录区域有关;B compartment对应染色质封闭区域,与基因荒漠、低转录区域有关^[77]。

生殖细胞经常会呈现独特的染色质结构。比如,小鼠晚期卵母细胞中除了TAD,还发现存在一种与H3K27me3标记区域高度相关的、不依赖cohesin的自相互作用分区,被称为多梳关联域(polycomb associating domain, PAD)^[78]。PAD被证明与卵子基因沉默相关^[78]。过去认为小鼠成熟精子中的染色质高级结构和体细胞相似,可以检测到广泛的TAD结构。但最近的研究指出,先前的结果可能受到了体细胞来源的游离染色质的污染。去除污染后,在小鼠的成熟精子中没有检测到TAD^[79]。此外,爪蟾和人类的成熟精子中也没有检测到TAD,但存在更长距离的顺式相互作用,后者可能是染色质受到鱼精蛋白紧密包装的结果^[80]。在小鼠中,受精卵和2细胞胚胎中loop、TAD和compartment都很弱,并在植入前发育过程中逐渐重建^[73](图1)。目前尚不清楚为什么TAD和compartment在受精后会变得高度松

弛。在人类胚胎中, TAD形成所需的CTCF在ZGA之前表达量很少^[74], 这提供了一个可能的解释。然而, 在小鼠和果蝇中^[70,73,81]CTCF在整个早期发育阶段均高度表达。小鼠胚胎在2细胞时期开始重建TAD, 这一过程不依赖于ZGA^[81]; 人胚胎在8细胞期开始重建TAD, 这一过程依赖于ZGA期间的CTCF表达激活^[74]。然而, CTCF缺失的小鼠胚胎可以发育到囊胚晚期, 并且桑椹胚时期的转录基本不受影响^[82], 这表明CTCF介导形成的loop和TAD在小鼠胚胎最初几个细胞周期中对转录并不是必需的。在果蝇胚胎中, 异染色质核心蛋白1a(heterochromatin protein 1a, HP1a)对于B compartment的从头建立至关重要。ZGA发生前, HP1a在染色质上的富集程度低, 并随着发育程度逐渐提高, 这或许可以解释为什么在果蝇早期胚胎中compartment处于不成熟的状态^[83]。

有趣的是, 在mESC中去除cohesin可解除对次要ZGA基因的抑制, 在供体细胞中预先去除cohesin会促进SCNT发生次要ZGA并提高发育成功率^[84]。这些结果提示松弛的染色质结构可能有助于促进基因组激活。此外, 通过过表达*Kdm4d*来去除体细胞的H3K9me3, 可促进H3K9me3标记的TAD的消解, 并提高SCNT胚胎的发育潜力, 这可能是通过释放超级增强子激活*Zscan4*实现的^[74]。这种松弛的染色质三维结构的功能仍然有待进一步研究。

DNA复制具有高度稳定的细胞特异性时间顺序, 被称为复制时序(replication timing, RT)。复制时序与区域性染色体状态, 尤其是三维基因组结构密切相关。通常来说, DNA早期复制区域与A compartment相对应, 而晚期复制区域与核纤层相关域(lamina-associated domain, LAD)和B compartment相对应^[85,86]。在2细胞期之前, 小鼠胚胎基因组已经建立了早期复制区域和晚期复制区域, 但此时两者的差异不明显。2细胞胚胎中, 父母本基因组的复制时序存在差异, 并在8细胞期后逐渐趋于一致, 这可能和父母本基因组不同的组蛋白修饰有关^[86,87]。在2细胞期, 卵母细胞中被PRC修饰的母源染色质尽管具有典型的晚期复制区域特征, 但却经历了早期复制^[87]。从4细胞期开始, 小鼠胚胎基因组早期和晚期复制区域的差异更加显著, 与同时期发生的A/B compartment形成相吻合^[87]。在ZGA期间, RNA聚合酶II在DNA上的定位和转录的发生有助于确保RT分化的准确性。晚期复制区域比早期复制区域更容易发生DNA断裂, 使得晚期复制区域的基因在发育过程中

具有更高的突变率, 这可能是复制胁迫条件下复制起始位点减少导致的^[86]。

2 ZGA时期在RNA水平的调控

2.1 mRNA翻译调控

完全生长的初级卵母细胞(full-grown oocytes, FGOs)会进入转录沉默状态, 直到受精后ZGA发生时基因组才会被重新激活^[88]。在这段转录沉默的时期, 翻译调控起着至关重要的作用。用环己亚胺(cycloheximide, CHX)抑制小鼠卵母细胞或早期胚胎的翻译会导致严重的发育停滞^[89]。用CHX处理小鼠或人类1细胞胚胎同样导致ZGA和母源mRNA清除严重受损^[90,91], 这些结果显示了翻译调控对于ZGA的重要性。在小鼠卵母细胞中, 大量mRNA直到减数分裂恢复后才进行翻译, 表明该时期存在丰富的转录后调控^[92]。这些翻译滞后的RNA通常被称为休眠mRNA (dormant mRNA)。位于3'非翻译区(3'-UTR)的细胞质多聚腺苷酸化元件(cytoplasmic polyadenylation elements, CPEs)在休眠mRNA的翻译调控中起到重要作用。CPE结合蛋白1(CPE binding protein 1, CPEB1)可以与CPE结合, 在FGOs中促进poly(A)尾的脱腺苷化, 导致翻译抑制。在减数分裂恢复过程中, CPEB1被磷酸化, 磷酸化的CPEB1能够促进poly(A)尾的腺苷化和翻译激活^[93]。在小鼠中, CPEB1是卵母细胞成熟所必需的^[94]。CPE与多聚腺苷化信号(polyadenylation signals, PAS)能够协同调节mRNA poly(A)尾长度进而调控翻译。poly(A)尾对调控mRNA稳定性和翻译效率至关重要^[95], 在多个物种的卵子向胚胎转换(oocyte-to-embryo transition, OET)过程中发挥作用^[96]。斑马鱼的miR-430通过促进poly(A)尾脱腺苷化确保母源mRNA清除^[97]。斑马鱼、爪蟾和小鼠的早期胚胎中, TUT4/TUT7催化短poly(A)尾的mRNA发生末端尿苷化是母源mRNA清除所必需的^[96,98]。在小鼠卵母细胞中, 母源蛋白BTG4或CNOT6L(脱腺苷化复合物CCR4-NOT的关键组分)缺失导致母源mRNA清除失败和早期胚胎发育停滞^[99]。

2.2 RNA修饰

N⁶-甲基腺嘌呤(m⁶A)是真核生物mRNA中丰度最高的修饰, 并广泛参与调节RNA的剪接、转运、降解以及翻译等过程, 被认为在配子发生和胚胎发育中发挥重要的调控作用^[100]。在卵母细胞中, 约40%的在受

精后被降解的母源mRNA富集m⁶A修饰. 敲除RNA甲基转移酶组分*Kiaa429*引起GV卵母细胞m⁶A水平剧烈降低, 富集m⁶A的转录本的丰度也显著降低, 表明m⁶A维持卵母细胞中母体RNA的稳定性^[101]. m⁶A修饰的转录本往往是miRNA的靶标, 且m⁶A修饰的母源转录本比m⁶A修饰的ZGA转录本更倾向于被miRNA结合并被清除^[102]. 在ZGA期间, 约50%的转录本富集m⁶A修饰, 阶段特异性转录本如*Zscan4*具有更高的m⁶A水平^[103,104]. 抑制RNA甲基转移酶METTL3可显著降低转录本m⁶A水平, 导致胚胎2~4细胞期退出延迟和囊胚形成率下降^[102,103]. 抑制METTL3不影响2细胞期转录本水平, 但短寿命转录本的RNA丰度下降推迟, 表明m⁶A促进了2~4细胞期特异性转录本的及时降解^[103]. 在类2细胞期细胞系(2C-like cell)中, m⁶A标记同样富集于阶段特异性转录本, 抑制METTL3和m⁶A读取蛋白IGF2BP2能够显著延迟类2细胞期状态的退出并提高类2细胞期细胞的数量^[104]. 此外, m⁶A修饰高度富集于逆转录转座子的RNA, 如卵母细胞中的MTA转座子和2细胞期特异表达的MERVL转座子. m⁶A可能对逆转录转座子的调节发挥重要作用^[103].

2.3 RNA剪接

剪接体是负责mRNA剪接和成熟的大分子核糖核蛋白复合体^[105]. 最近的研究表明, 剪接体还能直接控制转录的起始、延伸和终止^[106]. 在人类和小鼠中, 选择性剪接随着ZGA的发生变得高度活跃^[107,108]. 这种转换同时依赖于母源因子如BTG4, 以及子代ZGA表达产物^[107]. 选择性剪接可以产生有功能或无功能的转录本, 从而实现早期胚胎发育基因的阶段性特异性表达^[108]. 在小鼠早期胚胎中抑制关键剪接因子SF3B1导致胚胎在2~4细胞期阻滞^[107]. 在小鼠和人类中, 多种剪接因子在ZGA前表达较低, 提示早期胚胎中剪接体部分功能受到抑制^[109,110]. 在人类ESC(hESC)和小鼠ESC(mESC)中, 抑制剪接因子通常会上调ZGA期间表达的全能性标志基因, 如*Zscan4*, 并下调多能性标志基因, 如*Pou5f1*和*Sox2*^[109,110]. 通过抑制关键剪接因子SF3B1, 可以诱导hESC和mESC转变为全能性卵裂球样细胞(totipotent blastomere-like cell, TBLC)^[109,110]. 以上结果表明, 早期胚胎中的剪接体抑制参与了发育基因表达调控与全能性转变. 剪接体抑制选择性激活ZGA基因的机制尚不清楚. 相较多能性基因, ZGA期间表达的全能性基因通常序列很短, 且内含子很少^[111]. 因此, 一个可能的解释

是, 剪接体抑制会导致广泛的剪接抑制, 包括多能性相关基因的表达下降, 而对全能性相关基因的影响很小.

3 转座子与ZGA

在胚胎发育的早期阶段发生转座子的大规模激活^[112]. MERVL(murine endogenous retrovirus-L, 鼠内源性逆转录病毒-L)是一种内源性逆转录转座子, 被广泛认为是细胞全能性的标志之一^[113]. 在小鼠中, MERVL在2细胞期被DUX和OBOX4以冗余的方式激活表达^[114], 并在2细胞期结束后迅速沉默^[115]. *Obox4*或*Dux*敲除仍能产生可育小鼠, 而同时敲除*Obox4/Dux*则会显著降低囊胚质量和形成率^[114]. 敲低或抑制MERVL导致2细胞期特异基因的显著下调和1~2细胞期发育阻滞^[116,117]. 研究表明, MERVL的转录本可以通过改变染色质三维结构增加临近区域可及性^[117]. 同时, MERVL的LTR序列可作为启动子直接激活附近基因表达^[118]. 在小鼠胚胎中敲除MERVL导致周边区域的转录水平和染色质开放程度下调^[117]. 部分转录本可能会产生长非编码RNA(long non-coding RNA, lncRNA)^[119]. 近期研究发现, 次要ZGA产生的lncRNA的转录起始位点周围富集MERVL^[113], 抑制MERVL显著下调附近的lncRNA表达, 但对于蛋白质编码基因表达的影响不显著^[113]. 敲除MERVL附近的lncRNA会影响胚胎发育, 提示MERVL介导的次要ZGA期间的lncRNA的激活可能参与早期胚胎发育^[113].

长散在核元件1(long interspersed nuclear element-1, LINE-1)是哺乳动物中最丰富的逆转录转座子, 在ZGA过程中也发挥着重要作用. 在小鼠早期胚胎中, 进化上较年轻的LINE-1亚家族在2细胞期参与染色质重塑^[120]. 在受精后立即抑制LINE-1的转录会降低2细胞期的染色质开放程度, 干扰ZGA基因激活, 导致2细胞期阻滞^[120]. 而在2细胞期结束后持续激活LINE-1会阻碍8细胞期的染色质致密化, 导致胚胎发育失败^[120]. 同时, LINE-1转录本也可以作为核支架招募Nucleolin和Kap1蛋白, 促进rDNA转录, 抑制*Dux*表达, 进而结束2细胞期基因表达^[116]. 在ESC中抑制LINE-1导致多个2细胞期特异基因的表达上调, 表明LINE-1对于2细胞期的退出有重要作用^[116]. 综上, LINE-1对于ZGA具有多层次的调控机制.

4 ZGA相关转录因子

转录因子对ZGA扮演着不可或缺的角色. 潜在的

关键ZGA转录因子至少需要满足以下3个条件^[121]。首先, 这些转录因子在ZGA初期表现出高表达水平和丰富积累。某些重要的母源转录因子可能在卵子-胚胎转换(oocytes-to-embryo transition, OET)期间经历翻译水平的上调。其次, 这些转录因子能结合ZGA基因的启动子或增强子序列以激活ZGA。第三, 缺少这些转录因子会导致早期胚胎发育异常以及ZGA基因表达的失调。这里我们总结了不同物种(包括果蝇、斑马鱼、小鼠和人类)中调控ZGA基因表达的关键转录因子和其潜在的调控机制。

4.1 果蝇

在果蝇中, 次要ZGA发生在有丝分裂第8~13周期, 主要ZGA则出现在第14周期^[122]。在ZGA过程中, 染色质开放区域主要富集两种序列: “CAGGTAG”及其突变体, 统称为TAGteam位点^[123]; 以及GA序列。TAGteam位点可作为增强子, 与关键转录激活因子如锌指蛋白Zld结合, 促进早期胚胎基因的转录^[122,123]。Zld转录本在卵巢和卵子中高度积累, 但是在受精后1 h才开始翻译, 这是由于Pan Gu激酶磷酸化后不能对翻译抑制因子BRAT进行调控, Zld才能开始翻译^[124]。在主要ZGA启动之前, ZLD已经作为先锋因子结合了大量的顺式调控区, 从而维持染色质的开放状态^[125]。Zld缺失会导致果蝇胚胎出现严重的发育异常, 并导致大量ZGA基因的表达下调^[123], 其中80%的下调ZGA基因的增强子区域都含有TAGteam位点^[123]。GA序列可以与另外两个母源转录因子CLAMP和GAF结合^[126]。CLAMP和GAF能够提高染色质开放程度并激活ZGA基因^[127]。CLAMP缺失的胚胎无法形成正常的细胞骨架, 与ZLD缺失的情况一致^[128]。CLAMP促进ZLD在关键基因启动子上的占位, 与ZLD结合的ZGA基因表现出一定的重叠, 但也有所不同^[128]。随着胚胎的发育, ZLD对转录本的调控过渡到GAF^[129]。

4.2 斑马鱼

斑马鱼的ZGA通常发生在受精后约3 h, 是原肠形成和母源mRNA清除所必需的^[130]。在ZGA启动前, 母源转录因子Nanog、Pou5f3 (又称 Pou5f1)和Sox19b(又称SoxB1家族成员)翻译水平较高, 对启动ZGA和激活miR-430以诱导母源mRNA清除有重要作用^[130,131](图2)。在斑马鱼中, 敲低Nanog、Pou5f3和Sox19b会导致75%的ZGA基因异常表达, 胚胎阻滞在原肠胚之前^[130]。

其中, Nanog具有核心的作用。在次要ZGA发生时, Nanog能结合其中74%的次要ZGA基因, Pou5f3和Sox19b结合约40%的基因, 且miR-430与Nanog有最直接的结合, 其结合基序为CATT[T/G][T/G]CA^[130]。同时, 有研究显示Nanog、Pou5f3和Sox19b三个基因敲除(triple knock out, TKO)的胚胎中有37%的ZGA基因表达下调, 胚胎阻滞在受精后4 h, 即囊胚期的球形阶段^[20]。在TKO的胚胎中, 这些下调基因的启动子和基因体区域上RNA聚合酶II(RNA Pol II)缺失, 在活跃启动子上的H3K4me3 和 H3K27ac以及活跃增强子上的H3K4me1和 H3K27ac显著减少。其中, Sox19b对于招募组蛋白乙酰转移酶p300和组蛋白乙酰化酶Brd4到增强子和启动子上是必需的^[20]。然而, p300和Brd4的抑制剂处理表明组蛋白乙酰化本身似乎并不是ZGA期间染色质开放的主要驱动因素^[20]。

4.3 小鼠

在小鼠的早期胚胎发育过程中, 次要ZGA出现在1细胞晚期到2细胞早期, 而主要ZGA则在2细胞晚期发生^[132]。DUX(在人类中称为DUX4)是一种在胎盘哺乳动物中普遍存在的同源结构域(homeodomain)转录因子, 在ES细胞中过表达Dux能诱导产生2细胞样ES细胞, DUX被认定为可能的ZGA转录因子^[133,134]。DUX通常在次要ZGA期间特异表达并能够在主要ZGA时期激活一些基因。然而, Dux敲除小鼠仅有16.9%的主要ZGA基因受到影响, 部分敲除小鼠能够存活至成年^[135]。在ES细胞中, 过表达小鼠的Dux或者人类的DUX4能激活很多次要ZGA基因, 如Zscan4、Zfp352等。DUX还能结合到某些转座子上, 如逆转录转座子ERVL/HERVL和ERVL-MaLR^[133,134]。DUXBL在DUX表达后特异性地进入DUX结合区域, 与参与基因沉默的TRIM超家族成员TRIM24和TRIM33相互作用, 帮助胚胎退出DUX介导的2细胞期全能性状态^[136]。2细胞中DUXBL缺失会导致2细胞期转录本的持续表达, Duxbl敲除胚胎中Zscan4、Zfp352、MERVL的表达上调, 胚胎阻滞在4细胞及之前。此外, OBOX家族转录因子属于PRD-like同源异性转录因子(PRD-like homeobox TFs), 在卵子和早期胚胎中高表达^[137], 它们的DNA结合基序在ZGA基因的启动子上高度富集^[138]。小鼠中敲除母源表达的Obox1/2/5/7和合子表达的Obox3/4导致2~4细胞期阻滞, 32%的次要ZGA基因(如MERVL)和48%的主要ZGA基因(如Dppa2和Nr5a2)的激活会受到严重影响^[139]。这种

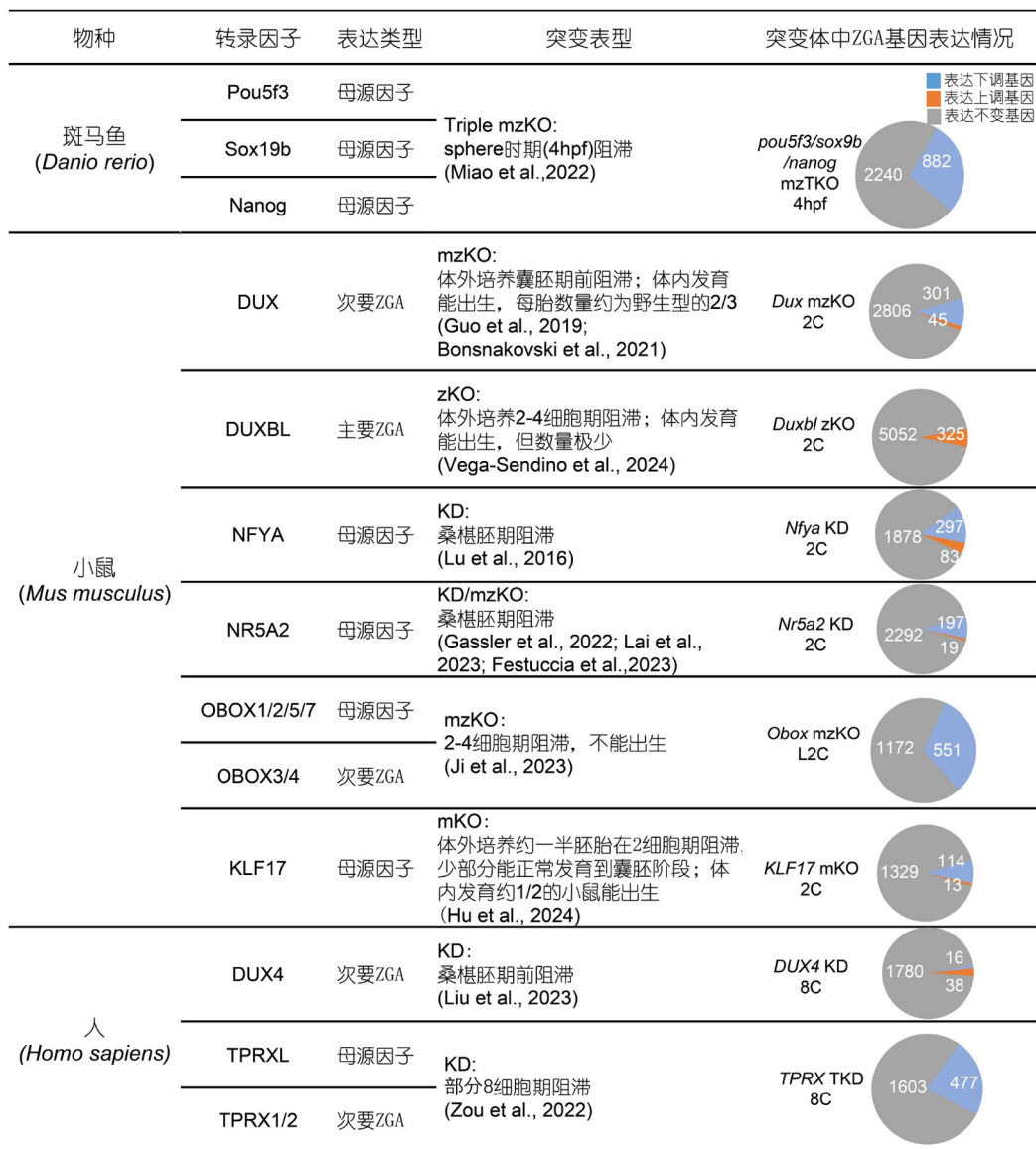


图 2 斑马鱼、小鼠、人中重要的ZGA转录因子。图中展示了不同物种中对ZGA有重要影响的转录因子及其表达类型、突变表型和突变体中ZGA基因表达情况。对于突变体中ZGA基因表达情况, 在扇形图中用蓝色表示表达下调的基因数量, 橙色表示表达上调的基因数量。需要注意的是, 不同论文中定义的ZGA基因存在差别, 因此不同论文的结果可能无法直接比较。mKO(母源敲除, maternal KO): 母本为纯合突变体, 子代为杂合突变体; zKO(合子敲除, zygotic KO): 母本为杂合突变体, 子代为纯合突变体; mzKO(母源合子敲除, maternal zygotic KO): 母本为纯合突变体, 子代为纯合突变体

Figure 2 Key ZGA Transcription Factors in Zebrafish, Mice, and Humans. The table displays transcription factors that significantly impact ZGA across different species, along with their expression types, mutant phenotypes, and ZGA gene expression in mutants. For the gene expression in mutants, a pie chart uses blue to indicate genes with downregulated expression, and orange for genes with upregulated expression. It is important to note that the definition of ZGA genes varies across different studies, so results from different papers might not be directly comparable. mKO (maternal KO): the mother is a homozygous mutant, and the offspring are heterozygous mutants. zKO (zygotic KO): the mother is a heterozygous mutant, the offspring are homozygous mutants. mzKO (maternal zygotic KO): the mother is a homozygous mutant, and the offspring are homozygous mutants

发育阻滞和ZGA基因表达受到的影响可以通过过表达母源*Obox1/2/5/7*或合子*Obox3*来挽救, 表明OBOX家族基因对于ZGA至关重要, 且具有很强的功能冗余性^[139]。OBOX能够结合在ZGA基因的启动子和增强子上, 帮

助打开染色质并招募RNA pol II来发挥功能^[139]。在ZGA过程中, OBOX能够及时地预配置pol II, 并将pol II招募到正确的ZGA基因上^[140]。在胚胎成纤维细胞中, *Obox1/3/6*过表达会激活ZGA基因^[137]。在小鼠ES细胞

中过表达 *Obox* 可以激活 ZGA 基因和 MERV1 [139]。OBOX 和 DUX 在调控不同的 ZGA 基因方面似乎各司其职, OBOX 同时负责调控次要和主要 ZGA 基因的表达, 而 DUX 则主要影响次要 ZGA 基因的表达 [140]。值得注意的是, *Obox4/Dux* 双敲低会导致囊胚发育缺陷 [114], 这表明 OBOX4 和 DUX 可能在胚胎发育中协同作用。

母源敲除 *Klf17* 会导致 9% 的 ZGA 基因表达下调, 约有 50% 胚胎在 2 细胞期阻滞, 而少部分胚胎能够正常发育到囊胚阶段 [141]。 *Klf17* 敲除导致的 ZGA 基因表达下调在很大程度上与 *Obox* 敲除后表达下调的 ZGA 基因重叠, 这些基因主要是低 CG 含量的, 而不重叠的部分则包括了具有较高 CG 含量的 ZGA 基因, 这暗示 KLF17 可能能够调控部分低 CG 含量及部分高 CG 含量的 ZGA 基因的表达。母源因子 TDP-43 可以帮助 pol II 在 ZGA 基因上结合, 敲除后小鼠胚胎阻滞在 2 细胞期, 伴随 23.7% ZGA 基因表达的变化 [142]。核受体转录因子 NR5A2 在 ZGA 时期高表达, 通过化学抑制剂处理后有 72% 的 ZGA 基因受到影响 [143]。 *Nr5a2* 敲除或敲低导致胚胎发育停滞在桑椹胚阶段, 而对 ZGA 基因表达影响较小 [144]。此外, NYFA、YAP1、RARG、DPPA2 和 DPPA4 与 ZGA 相关, 其中 DPPA2 和 DPPA4 在 2 细胞样 ES 细胞中被鉴定为重要的 ZGA 调控因子, 但是 KD 和 KO 实验证明它们对于 ZGA 和 2 细胞期之后的发育并不是必需的 [145]。

4.4 人类

在人 ES 细胞中, 过表达 DUX4 能够激活 8 细胞样状态下特异性基因的表达, 诱导产生 8 细胞样 ES 细胞 [146, 147]。在人类早期胚胎中, DUX4 的识别序列在 ZGA 基因的启动子上富集, 但是敲低 *DUX4* 只对 ZGA 产生微弱的影响 [148]。此外, DUXA、DUXB 与 DUX4 同属于 DUX 家族, 在人早期胚胎中也有表达, 属于 PRD-like 同源异型转录因子家族 [149]。在 293T 中, 有 DUX4 结合位点的基因中, 约 70% 的基因上 DUX4 结合位点的附近也会有 DUXA 的共定位, 在人成肌细胞中过表达 *DUXA* 会抑制 DUX4 靶向基因的表达 [150]。DUX4 能够通过 C-末端招募 P300/CBP 和 MED15, 进而招募 H3K27ac, 促进靶基因 *ZSCAN4* 的转录激活 [148]。PRD-like 同源异型转录因子 (PAIRED-like homeobox transcription factors) 在人早期胚胎中表达, 部分转录因子能够富集在 ZGA 基因启动子区域 [149]。TPRX1 被认为是 8 细胞样细胞 (8C-like cell) 的标志物 [146, 147, 151]。PRD-like 同源异型转录因子 TPRXL、TPRX1 和 TPRX2 在 ZGA 前后有高翻译水平,

TPRX1/2 或 *TPRXL* 敲低会导致约 15%~21% 的主要 ZGA 基因受到影响, 而 *TPRX1/2/L* 三个基因同时敲低会导致 31% 的主要 ZGA 基因受到影响, 胚胎阻滞在 4~9 细胞期, 这提示它们可能存在功能冗余 [91]。 *TPRX1/2/L* 三者之间合作调控 ZGA 基因表达的机制还需要更深入的研究。另一方面, 人类 ZGA 基因的启动子区域富含 Alu 元件 (也称为 EGA 富集的 Alu 基序, EEA 基序) [138, 152]。PRD-like 同源异型转录因子包括 OTX2、LEUTX、TPRX1、TPRX2 和 DPRX, 能够结合至这个基序并激活相关基因表达 [152]。有研究表明, 在人类 1 细胞胚胎中敲低 *OCT4* 会导致 25% 的 ZGA 基因表达下调 [153]。但是也有报道发现敲除 *OCT4* 之后, 胚胎在较晚时期才会受到影响, 表现为无法形成囊胚且缺乏谱系分化相关的特异性基因表达 [154]。此外, OCT4 蛋白在人类 8 细胞时期才会出现 [155]。这些结果提示 OCT4 的功能有待进一步研究。有趣的是, 在正常胚胎和孤雄胚胎中, ZGA 起始于 8 细胞期, 而孤雌胚胎的 ZGA 则起始于桑椹胚阶段 [156], 提示在 8 细胞期父源特异性表达的 ZNF675 和 LSM1 对 ZGA 有重要作用。

5 ZGA 期间的蛋白组

对于爪蟾 [157]、小鼠 [158]、人类 [159] 的配子和早期胚胎的蛋白质组研究表明, 早期胚胎中蛋白质组变化明显滞后于转录组和翻译组。在小鼠中, 转录组与翻译组在 ZGA 前后发生了剧烈变化, 但是从 FGO 到 8 细胞期的蛋白组都主要由 FGO 储存的蛋白质占据主导地位 [158, 160]。FGO 来源的蛋白质经常能稳定维持到囊胚期, 即使相应的转录本已降解 [160]。受精胚胎中同一时期的转录组和蛋白质组在初始阶段相关性很低, 但随着发育进程而逐渐增加 [158, 160]。这些结果表明蛋白质组的研究在早期胚胎中也具有重要意义。目前的质谱技术对于检测转录因子等低丰度蛋白还具有一定局限性, 难以准确比较这一类蛋白的丰度变化。

6 ZGA 与代谢

从受精卵到桑椹胚阶段, 小鼠、兔和猴胚胎主要利用来自输卵管液的丙酮酸和乳酸作为首选能量底物 [161]。在 2 细胞期胚胎中, 由于线粒体活性较低, 丙酮酸对于胚胎在 2 细胞期以后的发育以及 ZGA 激活至关重要 [161]。如果在培养基中添加葡萄糖和乳酸, 但未添加丙酮酸, 胚胎将会阻滞在 2 细胞期 [162]。受精后, 三羧酸循环 (tricarboxylic acid cycle, TCA cycle) 相关的酶短

暂进入细胞核,对表观遗传重塑以及ZGA至关重要^[163]。例如通过丙酮酸氧化产生的细胞核内TCA循环产物 α -酮戊二酸(α -ketoglutaric acid, α -KG)是组蛋白去甲基化所必需的^[163]。在小鼠2细胞期,H3K4me3标记会被组蛋白去甲基化酶KDM5A和KDM5B擦除,而 α -KG是这两种酶的辅助因子^[164,165]。在无丙酮酸的培养基中添加 α -KG能够挽救2细胞期的发育阻滞^[163]。除了对组蛋白甲基化的影响,在小鼠早期胚胎中, α -KG通过调节DNA去甲基化酶TET的催化活性和DNA甲基转移酶*Dnmt3a*的表达水平,增加5hmC/5mC的比例,从而促进囊胚期内细胞团的多能性^[166]。在人类胚胎干细胞中,增加 α -KG会降低DNMT3B的表达并触发多向分化^[167],但是对于ZGA的影响尚未有明确证据。此外,代谢过程还能通过调控组蛋白乙酰化来精确调控次要ZGA^[21]。NAD⁺通过与SIRT1合作去除组蛋白乙酰化,是次要ZGA退出所必需的^[21]。在人类胚胎中,补充NAD⁺能促进受精卵H3K27ac的擦除,有利于植入前的发育^[21]。在哺乳动物中,乳酸通过H3K18乳糖化调节主要ZGA的激活^[168]。人类和小鼠的主要ZGA发生时,早期胚胎的细胞核中高度富集乳酸,乳酸衍生的H3K18乳糖化在大多数ZGA基因的启动子区域富集^[168]。抑制乳酸的产生和摄取会导致胚胎发育停滞在2细胞期,主要ZGA无法进行以及H3K18ac的丢失,可以通过添加酰基辅酶A或过表达H3K18R突变体来挽救。这些证据表明,代谢在ZGA发生中也扮演着重要作用。最近有研究指出,当氧气浓度由生理氧水平(5% O₂)转换为大气氧水平(20% O₂)时,体外受精的胚胎会出现发育延迟的现象。胚胎氧气浓度的变化不会影响次要ZGA,然而约300个主要ZGA基因表达下调,2细胞期的新生成的RNA下

降,且氧敏感转录因子HIF-1 α 核定位减少。约17%的主要ZGA基因上存在HIF-1 α 的结合位点。用HIF-1 α 特异性抑制剂处理后,HIF-1 α 靶向的ZGA基因发生转录抑制^[169]。这种现象在牛和猪的早起胚胎中也是保守的^[169]。这些研究结果表明,氧气浓度可能会通过HIF-1 α 影响ZGA。

7 总结与展望

过去几十年来,我们对于受精后的第一个转录事件——ZGA的理解取得了快速进展。本篇综述中,我们主要讨论了表观遗传重编程如何为ZGA创造许可的染色质状态,关键转录因子(TFs)如何激活特定基因以开启下游的基因级联表达,以及在RNA水平对ZGA进行的调控。然而,与ZGA相关的许多关键问题仍有待回答。例如,还有哪些关键的ZGA转录因子尚待发现?ZGA期间的表观遗传因子和转录因子交叉对话(cross-talk)是怎样进行的?为什么ZGA转录因子表达谱和作用机制在进化过程中会在不同物种间发生重大分化?转座子元件与转录因子之间有何样的功能关系?哪些因素精确地控制了ZGA的发生时间?另一方面,样本稀缺性仍是早期胚胎研究的主要障碍。低样本量(low-input)单细胞测序技术的优化对未来的早期胚胎研究十分重要。同时,建立合适的体外ZGA模型有助于克服样本稀缺的难题。目前,小鼠类2细胞期细胞(2C-like cell)已被证明对于研究次要ZGA极具价值,但对主要ZGA的研究仍然缺乏有效的体外模型。建立这些体外模型将有助于进行大规模生化分析和遗传筛选,帮助我们揭示与ZGA的本质密切相关的科学问题。

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Summary for “开启生命程序：合子基因组如何启动?”

Kickstarting the program of the life: How is the zygotic genome activated?

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In all species studied so far, the zygotic genome undergoes a period of transcriptional silence after fertilization. Zygotic genome activation (ZGA) is the first transcriptional event post-fertilization, marking the onset of the embryonic gene expression program and the establishment of totipotency. Defects in ZGA usually severely impact embryonic development, even leading to lethality. The exact timing of ZGA and the biological events during this period vary among species. Due to the scarcity of early embryo materials, the mechanisms underlying ZGA have historically been difficult to elucidate. With the rapid development of low-input and single-cell analysis technologies, our understanding of ZGA has significantly advanced in recent years. Studies have revealed the dynamic landscapes of the epigenome, transcriptome, proteome, and metabolome during ZGA. Activating chromatin modifications, such as H3K27ac and H3K4me3, and repressive chromatin modifications, such as DNA methylation, H3K9me3, H3K27me3, and H2AK119ub, undergo dramatic reprogramming in early mammalian embryos to accommodate active transcription during ZGA. Histone variants, such as H3.3 and H2A.Z, also participate in gene regulation and epigenetic reprogramming in early development. The widely relaxed higher-order chromatin structure following fertilization is a hallmark of early embryo, providing a unique environment for gene expression. Multi-level RNA regulation, such as RNA modification, splicing regulation and translational regulation, plays crucial roles in gene expression regulation. Transposable elements, such as MERVL and LINE, are massively activated during early stages of mammalian embryonic development, influencing gene expression regulation and epigenetic reprogramming. Key transcription factors, either deposited by oocytes or transcribed at the early phase of ZGA, function as “specifiers” to drive the onset of ZGA by activating the expression of selected targets. Reprogramming of the proteome also differs substantially from those of the transcriptome and translome, suggesting extensive post-translational regulation. Metabolites also influence ZGA by participating in epigenetic reprogramming, which is a fast-developing research direction.

Despite the exciting progress in unveiling the molecular principles underlying ZGA, many critical questions regarding ZGA remain unanswered. For example, what determines the timing of ZGA across species? How are some epigenetic marks at specific sites retained during epigenetic reprogramming and inherited across generations? What are the upstream factors that prime ZGA? How do epigenetic factors and transcription factors crosstalk during ZGA? Why have ZGA transcription factors diverged significantly across species during evolution? On the other hand, the scarcity of samples is a major obstacle in early embryo research. The development of low-input and single-cell analysis technologies remains crucial for the field. The establishment of appropriate in vitro ZGA systems, such as 2C-like cells and 8C-like cells, is also essential for dissecting the molecular circuitry governing ZGA. In this review, we will first summarize the current progress in understanding the epigenetic reprogramming events during ZGA in different species. We will also review the key factors controlling the timing and specificity of ZGA, and the multi-dimensional molecular reprogramming in ZGA. Finally, we will discuss the key unresolved questions in the field of ZGA research that await answers.

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